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Foliar spray of moringa leaves extract enhanced the chemical contents of Lemon Bee balm plant (*Monarda ciriodora* L.)

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Abstract

Monarda citriodora L. plant is an important aromatic and medicinal plant (Fam. Lamiaceae). It is economical importance due to its rich in Thymol compound in the volatile oil. The dependency on the use of chemical fertilizer as a source of plant nutrients and their high cost is further associated with land soil degradation and environmental pollution. The objective of this study was to investigate the tested of three levels of chemical fertilizers (recommended dose 100% NPK, 300:200:100 kg/fed., 75% and 50% NPK). As well as foliar spray of moringa leaves extract (MLE) as growth promoting at 0.0, 1:40, 1:30 and 1:20 and their interactions between them on essential oil production, total phenolic content, total flavonoids content and antioxidant activity of *Monarda citriodora* L. plant. The highest level of NPK 100% (RD) improved the most chemical contents followed by 75% and then 50%. The different concentrations of MLE significantly increased the chemical contents as compared to the control treatment. These constituents increased gradually with increasing the concentration of MLE up to (1:20). Under the same level of NPK, MLE application increased the chemical constituents comparing to NPK without MLE spray. Also, it was observed that the use of the natural extract (MLE) combined with low levels of chemical fertilization lead to increase these constituents compared to high levels of the chemical fertilization (NPK) without spraying the MLE extract. So, applying the natural extract of MLE can be partial substitution of the chemical fertilizers and produced a high quality of *Monarda citriodora* L., plants.

Keywords: *Monarda citriodora* L., NPK, Moringa leaves extract, Essential oil, Total phenolic, Total flavonoids, Antioxidant activity

Introduction

Monarda citriodora is an annual herb, belongs to the Lamiaceae family, and is commonly known as lemon bee balm, lemon bergamot, horsemint, purple horse mint, plain horse mint or lemon mint. The genus of *Monarda* is represented by 15 species native to the United States, Canada and Mexico and it is also cultivated in Europe and Asia [1, 2]. Monarda is grown as an ornamental, aromatic and medicinal plant throughout most regions of the world [1, 3, 4]. The best known species of this genus are; *Monarda didyma* L., *M. fistulosa* L. and *M. citriodora* [5].

The edible parts of plant are leaves, it is widely used as a good flavour and garnish agent in salads, soft drinks, bakery, meat products and salad, seafood, chicken, and meat dishes, cakes, sauces, and pies. It's also used sometimes in wines and liqueurs [4, 6, 7].

Traditionally, leaves have been used to make tea which is used to treat digestive gas, respiratory disorders, diuretic, febrifuge, diaphoretic, antirheumatic, carminative, sedative, stimulant, upset stomach, as a cold and cough remedy, and as a pleasant beverage [4].

Monarda citriodora L. has a rich source of volatile oil, where thymol reported 70.6%, *p*-cymene 10.6%, carvacrol 6.1%, terpinen-4-ol 1.2% accounted for 95.9% of the oil [3]. Under Egyptian conditions ,the constituents of essential oil in *Monarda citriodora* were thymol that was the major compound ranged (32.73 – 63.88 %), followed by carvacrol (6.54 - 29.56%), which constituted almost 80 % of the essential oil, then p-cymene (1.24-17.72 %) and γ-

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terpinene (0.37-19.6%) [8].

Essential oil of *Monarda citriodora* L., has antiseptic properties and is now used in many of pharmaceutical industries and modern commercial mouthwash formulations [3, 9, 10]. Also, essential oil of *Monarda citriodora* L. has strong antibacterial and antifungal properties, antioxidant activity, and against various human pathogens [2, 3, 9, 11]. Also, It is clear that *M. citriodora* var. *citriodora* oil is a preservative in against free radical-mediated deterioration of lipid-rich , foods, cosmetics and pharmaceuticals [3] and it is insect repellent and in perfumery industry [12].

Phenolic and flavonoids compounds are the most important groups of secondary metabolites and bioactive constituents in plants and health of human [13]. These compounds are very important to regulate the growth and development of plants by their functions in many processes such as photosynthesis and reproduction [14].Also, phenolic and flavonoids are very important in medicinal and commercial value of edible and medicinal plants [15, 16]. Phenolic and flavonoids compounds in many foods play an important role in human health, anti-aging, reducing the risk of cancer and antioxidant substance [13].

Several authors reported that NPK fertilizer significantly enhanced the production of essential oils and active ingredients of some medicinal and aromatic plants, such as *Monarda citriodora*, [8, 17]. *Ocimum basilicum* L. [18, 19] and *Satureja hortensis*, [20].

However, using intense chemical fertilizers cause serious problems on human health by pollution of the whole environmental conditions (soil, air and drainage water). Thus, efforts are being made to provide alternative safe natural sources of plant nutrients.

For avoiding the excessive use of the mineral chemical fertilizers , it could be that replaced partially or completely by some natural bio stimulants such as plants extracts that characterized by their richer contents with nutrient elements, growth regulators, antioxidants and vitamins were investigated for applied on the plants.

Moringa leaves extract was reported to increase the percentage and chemical contents of some medicinal and aromatic plants. *Moringa oleifera* L. belongs to moringaceae family. Moringa leaves extract is a rich source of macro and micro nutrients, amino acid, vitamin E, ascorbic acid, phenolic and flavonoids compounds and growth regulating hormones like zeatin [21, 22]. Thus it the potential to promotes plant growth, it can be used as a natural plant growth. Zeatin is one of the phytohormons form of the most common forms which is naturally occurring cytokinin that not only promotes the growth of plants by facilitating cell division and cell elongation as well as its anti-aging potential and protective effects in plants [23, 24]. The potential of MLE when applied through seed or plant foliage have been shown to improve the plant tolerance to abiotic stresses, including salinity, enhanced antioxidant levels and activated plant defence system, increased levels of plant secondary metabolites [25-27].

Some authors recorded significant enhancement by foliar application of moringa leaves extract on chemical constituents (volatile oil percentage, total flavonoids and total phenolic content) of Jojoba and *Narcissus tazetta* L. plants [28,29] respectively, fennel plant [30], *Salvia officinalis* L. [31] and *Pelargonium graveolens* [32]. The interaction between NPK and MLE increased oil percentage of dill plant [33] and *Pelargonium graveolens* L. plant [34]

The aim of this study is to investigate the effect of different level of NPK, foliar application of the natural extract of moringa leaves and interaction between them on chemical constituents of *Monarda citriodora* L. plant.

Materials and methods

This work was carried out during two successive seasons 2016/2017 and 2017/2018 at the Experimental Farm of Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, and Medicinal and Aromatic Plants Research Department, National Research Center, Dokki, Giza, Egypt.

This experiment was conducted to evaluate the role effect of partial substitution of NPK by using MLE on the percentage and compositions of volatile oil as well as chemical constituents of *Monarda citriodora* L. plant.

Plant material

The seeds of *Monarda citriodora* L. plant were obtained from Dr. Helmut Junge, ABiTEP GmbH,

Berlin, Germany, providing the plant material, via Jelitto GmbH, Germany.

Cultivation procedures and maintenance

The seeds were sown in plastic bags on the last week of September during the two successive seasons in a mixture of sand and peat moss (1:1) and located in a nursery inside the greenhouse. After two months from swing, when the seedling reached 12-15cm in height, were transplanted on the last week of November to the previously prepared plastic pots (35 cm) filled with 7kg soil at the experimental field in the two successive seasons (two plants per pot then it was thinned for one plant). Recommended dose (RD) was ammonium sulphate (20.5% N) 300 kg/fed. as a source of nitrogen, calcium superphosphate (15.5% P_2O_5) 200kg/fed. as a source of phosphorus and potassium sulphate (48% K_2O) 100 kg/fed. as a source of potassium. Calcium superphosphate was added during soil preparation.

The experiment included 12 treatments, arranged in a split plot design. Chemical fertilizers at three levels, 100% (RD), 75% and 50% of (RD) occupied the main three groups of pots (main pots), while the natural extract treatments of MLE, M1 1:40, M2 1:30, M3 1:20 (one crude extract diluted with 40, 30 and 20 times water respectively, as well as control treatment (water only) occupied the sub pots. Nitrogen and potassium fertilizers were added to the soil as side dressing and divided into two equal portions, the first addition was after one month from transplanting and the second one was after the first cut. (MLE) was foliar sprayed four times during the two growing season. The first one was after one month from transplanting date, second addition was after one month from the first addition, while third and fourth spraying was carried out after the first cut.

Preparation of Moringa leaves extract (MLE)

The fresh leaves of *Moring oleifera* plant were obtained from Egyptian scientific Society of Moringa, National Research Centre, the leaves were free from any form of pest infestation and disease then, cut into small pieces and air dried. For extraction, the powdered sample (20g) was soaked in ethanol 80% and the mixture was stirred occasionally by using a rotary Shaker .Extract was purified by filtering twice through filter paper (whatman No.1). After purification, the extract was diluted with distilled water to obtain the required concentrations [21]. MLE M1 1:40, M2 1:30, M3 1:20 (one crude extract diluted with 40, 30 and 20 times water respectively, as well as control treatment (water only).

Harvesting

The plants were harvested twice yearly by cutting the aerial parts of each plant 10cm above the soil surface leaving 2-3 branches with some leaves for regrowth. The first cut was harvested on the first of March and the second one was carried out in mid-May for both seasons.

Data recorded

1. Essential oil isolation and determination

Samples of fresh aerial parts for each replicate (100g of each sample) of *Monarda citriodora* L. and were subjected by hydro-distillation for 3 hours using Clevenger-type apparatus to extract and determine oil percentage according to Guenther [35]. The essential oil content was calculated as a relative percentage (V/W). The essential oil extracted from *Monarda citriodora* L. herb which were collected during the first and the second cuts during both seasons for each treatment dried over anhydrous sodium sulphate until identify the chemical constituents.

2. Analysis of essential oil

The chemical constituents of the essential oil for samples of each treatment were analyzed using gas chromatographic (GC). The use of GC in the quantitative determinations was performed using the methods described by Mihajilov-Krstev *et al*. [36].

Conditions of GC analysis

The GC analysis of the essential oil samples were carried out in the second seasons using gas chromatography instrument stands at the Laboratory of Medicinal and Aromatic Plants, National Research Centre with the following specifications.

Instrument: capillary GC-2010 plus Gas Chromatographs (Shimadzu Corp., Japan), coupled with a Shimadzu FID 2010 Plus detector (Flame Ionization Detector). The GC system was equipped with a Stabilwax column (30 m x 0.25 mm i.d., 0.25 μm film thickness). Analysis were carried out using helium as carrier gas at a flow rate of 1.0 mL/min at a split ratio of 1:10 and the following temperature program: 40º C for 1 min; rising at 4.0º C/min to 150º C and held for 6 min; rising at 4º C/min to 210º C and held for 1min. The injector and detector were held at 210º C and 250º C, respectively. Diluted samples

(1:10 hexane, v/v) of 0.2 μL of the mixtures were always injected. Most of the compounds were identified using GC standards. The obtained chromatogram and analysis report for each sample were analyzed to calculate the percentage of the main volatile oil components. The area of each peak was first calculated by an automatic integrator. The areas were then summed, and the total area of the peaks represented the whole sample. The percentage of each component was the ratio between its peak area to the total peak areas, multiplied by 100.

3. Determination of total phenolic content (mg/g)

The total phenolic content was determined in extract of dry herb of each treatment collected at both cuts in two seasons using the folin-Ciocalteu's reagent according to Singleton *et al*. [37].

4. Determination of total flavonoid content (mg/g)

The content of total flavonoids in the extracts of dry herb was determined by the aluminium chloride using spectrophotometric method according to Quettier *et al.* [38].

5. Determination of free radical scavenging activity %

The free radical scavenging activity was assessed in extract of dry herb by the standard method [39], adopted with suitable modifications [40].

Statistical analysis

Data of each season were statistically analysed by ANOVA test (MS DOS/ Costat Exe Program) according to Gomez and Gomez [41]. The LSD (least significant difference) level at 5% was used to compare the means value according to Snedecor and Cochran [42].

Results

1. Essential oil percentage.

The essential oil percentage in the fresh herb of *Monarda citriodora* L., are presented in Table (1). In the first and second cuts during the first season, chemical fertilization was most effective when applied at NPK 75% of RD which recorded the highest value of volatile oil percentage (0.22% and 0.82%) followed by (RD) NPK 100% (0.19% and 0.72%), then half dose NPK 50% which gave the least values (0.15% and 0.64%) while, at the second season, the mean values of volatile oil % were (0.24% and 0.89%), (0.22% and 0.81%) and (0.18% and 0.69%) at the two cuts for the same previously mentioned treatments, respectively.

The differences between NPK treatments were significant at two cuts of both seasons. Also, it was observed that the second cut had higher essential oil percentage than the first cut. This trend has been noticed in the two cuts during two seasons. Similar results were also reported by Mostafa *et al.* [43] on *Dracocephalum moldavica* plants and Verma *et al.* [44] on *Stevia rebaudiana* plant. They found that 100% NPK (RD) increased the volatile oil production

As for the effect of spraying natural extract of MLE on volatile oil percentage of *Monarda citriodora* L., plant, data in Table (1) revealed that spraying plants with MLE at all concentrations increased essential oil percentage comparing to unsprayed treatment in both cuts of both seasons. It is clear that volatile oil increased gradually with increasing the concentration of MLE. So, the concentration of MLE at 1:20 increased the volatile oil percentage by (53.33% and 38.89%) in the first cut, while these increments were (47.46% and 33.82%) in the second cut during two seasons, respectively compared to untreated plants.

Data presented in Table (1) show that the interaction treatments had no significant effect (except the 2nd cut of the 2nd season) on volatile oil percentage.

Under the same NPK level, the interaction with any concentration of MLE application increased volatile oil percentage comparing to NPK fertilized plants without spraying natural extract of MLE. The highest values of volatile oil percentage in fresh of herb were observed by using the high concentrations of MLE (1:20) companied NPK (75%). Also, it was noticed that low levels of NPK proved to be more effective in enhancing the percentage of volatile oil by spraying natural extracts of MLE at high concentration (1:20).The interaction between the levels of NPK and foliar spray of natural extract of MLE was insignificant during the two cuts except the second cut of the second season.

Table 1. Effect of moringa leaves extract, chemical fertilizers and their interactions on essential oil percentage of *Monarda citriodora* L. plants

				Essential oil (%)				
				First season				
	First cut				Second cut			
Ext. Conc.	Chemical fertilizer rates (NPK)							
	100%	75%	50%	Mean	100%	75%	50%	Mean
Control	0.15	0.18	0.13	**0.15**	0.57	0.66	0.54	**0.59**
M1 (1:40)	0.18	0.21	0.14	**0.18**	0.69	0.76	0.59	**0.68**
M2 (1:30)	0.19	0.24	0.16	**0.20**	0.73	0.87	0.68	**0.76**
M3 (1:20)	0.25	0.26	0.18	**0.23**	0.89	0.99	0.73	**0.87**
Mean	**0.19**	**0.22**	**0.15**		**0.72**	**0.82**	**0.64**	
LSD(0.05)	Chem.			**0.02**				**0.02**
	Ext.			**0.02**				**0.03**
	Chem. X Ext.			**N.S**				**N.S.**
				Second season				
Ext. Conc.	First cut				Second cut			
	100%	75%	50%	Mean	100%	75%	50%	Mean
Control	0.19	0.20	0.16	**0.18**	0.65	0.76	0.63	**0.68**
M1 (1:40)	0.20	0.23	0.17	**0.20**	0.78	0.85	0.64	**0.76**
M2 (1:30)	0.21	0.25	0.19	**0.22**	0.86	0.93	0.69	**0.83**
M3 (1:20)	0.27	0.28	0.20	**0.25**	0.95	1.01	0.78	**0.91**
Mean	**0.22**	**0.24**	**0.18**		**0.81**	**0.89**	**0.69**	
LSD(0.05)	Chem.			**0.04**				**0.03**
	Ext.			**0.04**				**0.02**
	Chem. X Ext.			**N.S.**				**0.04**

Ext.Con. (Extract Concentration) M (Moringa leaves extract) Chem.(Chemical fertilizer rates, NPK) N.S. (Non-significant)

2. Essential oil (EO) constituents

The effect of interaction between the different levels of NPK and foliar application of MLE at different concentrations on the EO compositions in fresh herb of *Monarda citriodora* L. plant are shown in Table (2).The values of components, thymol as the major component (22.2-79.0%) followed by γ-terpinene as the second component (5.3-25.9%) and carvacrol (5.8 -18.6%) and then ρ-cymene (3.4-13.5%). Other components arranged in descending order, α-terpinene (0.3-7.8%) and α-phellandrene (0.5- 6.1%) as well as α-pinene (0.9- 4.2%) and trans-sabinene hydrate (0.7-3.6%). The total identified components in the EO accounted (91.8-98.3%) for all components with different treatments.

Thymol component

Comparing the interaction between the different levels of NPK and concentrations of MLE, the highest content of thymol compound (79.0%) were recorded with 75% NPK plus MLE at high concentrations (1:20) followed by application of 100% NPK plus MLE at (1:20) which recorded (65.8%).

γ-Terpinene, Carvacrol and p-Cymene

Concerning, the effect of the combination between NPK and MLE, the results indicated that the highest content of γ-terpinene (25.9%), was gained from 100% NPK treatment without MLE spray, followed by 100% NPK plus MLE at concentration (1:30) which gave (21.2%). Also, maximum relative percentage of carvacrol (18.6%) was obtained when plants fertilized with 100% NPK plus MLE at the lowest concentration (1:40), while the maximum value of ρ-cymene content (13.5%) occurred when applying 75% NPK with MLE at the medium the concentration at (1:30).

From the obtained results, it could be noticed that the major compounds, thymol, γ-terpinene and carvacrol were significantly affected by interaction with all the treatments of NPK and MLE. It can be observed that increment of thymol content with some treatments leads to decrease γ-terpinene, carvacrol and p-cymene contents. These results may be attributed to the fact that some conditions accelerate the thymol compound against decrease of other components. These findings agree with Piccaglia [45] who reported that differences in the ratio between the components of thymol, carvacrol, γ-terpinene and p-cymene in the essential oil of *Satureja montana* were observed which could be attributed to the effects of environmental conditions. Also, on *Monarda citriodora* L. plant was mentioned that the different levels of nitrogen increased thymol content and decreased carvacrol [8].

Table 2. Effect the interaction between moringa leaves extract and chemical fertilizers on the essential oil components (%) of *Monarda citriodora* L. plants at the second cut of the second season (2017/2018).

No	Components %	RT.	Class	Chemical fertilizer rates (NPK)											
				100%				75%				50%			
				0	M1	M2	M3	0	M1	M2	M3	0	M1	M2	M3
1	α-Thujene	6.45	MH	1.2	1.0	1.2	0.6	0.8	1.0	1.2	----	0.5	1.1	0.6	0.3
2	α-pinene	6.62	MH	4.2	3.3	4.0	1.9	2.7	3.1	4.0	0.6	1.5	3.6	2.0	0.9
3	α-Phellandrene	10.7	MH	5.2	6.1	6.0	2.7	3.9	5.2	5.9	0.5	3.3	6.0	3.7	1.8
4	α-Terpinene	11.23	MH	6.3	6.3	6.5	2.9	4.0	5.8	7.8	0.3	3.7	5.8	3.6	1.8
5	β -Cymene	11.8	MH	1.9	1.7	1.7	0.7	1.3	1.6	1.7	----	0.9	1.8	1.1	0.8
6	ρ -Cymene	13.47	MH	5.5	9.0	11.0	4.5	3.4	6.4	13.5	----	6.8	8.1	6.6	6.5
7	γ-Terpinene	14.5	MH	25.9	19.0	21.2	8.0	14.3	18.4	21.0	5.3	11.1	20.3	13.9	5.3
8	Trans-Sabinene hydrate	20.40	OM	3.5	2.7	2.5	1.0	1.5	2.1	2.4	1.6	1.8	3.6	2.4	0.7
9	4-Terpineol	25.36	OM	0.9	1.0	1.0	0.4	0.5	1.5	0.8	0.9	0.7	1.0	0.7	1.2
10	α-Terpineol	25.68	OM	3.2	1.1	0.7	0.6	0.8	0.6	0.3	0.7	0.6	0.7	0.5	2.8
11	Carvacrol methyl ether	39.19	OM	1.0	1.2	0.5	0.2	0.5	0.8	0.7	0.7	0.7	1.3	0.2	0.6
12	Thymol	46.57	OM	28.6	23.1	23.5	65.8	52.3	36.5	22.2	79.0	56.6	29.9	49.8	52.1
13	Carvacrol	47.38	OM	7.0	18.6	13.0	5.8	9.6	13.0	11.3	8.7	8.5	12.4	10.9	17.0
MH = Monoterpene hydrocarbons				**50.2**	**46.4**	**51.6**	**21.3**	**30.4**	**41.5**	**55.1**	**6.7**	**27.8**	**46.7**	**31.5**	**17.4**
OM =Oxygenated monoterpenes				**44.2**	**47.7**	**41.2**	**73.8**	**65.2**	**54.5**	**37.7**	**91.6**	**68.9**	**48.9**	**64.5**	**74.4**
Total identified				**94.4**	**94.1**	**92.8**	**95.1**	**95.6**	**96.0**	**92.8**	**98.3**	**96.7**	**95.6**	**96.0**	**91.8**

The results in Table (3, 4 and 5) show the effect of different levels of chemical fertilizers (NPK), natural extract of moringa leaves extract (MLE) and interaction between them on the total content of phenolic, flavonoids and free radical scavenging activity%.

3. . Total phenolic content (TPHC) mg/g

The effect of chemical fertilizers treatments on the TPHC of *Monarda citriodora* L. plant is shown in Table (3). The TPHC was influenced significantly by the application of various levels of chemical fertilizers (NPK). The maximum values of the TPHC (12.04, 15.07 for 1st season against 11.95, 15.65 mg/g for the 2nd one) were obtained with RD of NPK (100%) followed by 75% and then 50% NPK. On the other hand the lowest mean values were (9.76, 11.67) and (9.52, 12.38) mg/g was obtained from plants fertilized with 50% NPK at both cuts of the two seasons, respectively. The differences between the three levels of NPK were significant, for both cuts in the two seasons.

As for the effect of foliar spray of natural extract MLE on TPHC content, the results in the same Table (3) show that all the used doses of MLE enhanced the phenol content compared to the control plants. These increments were recorded gradually with increasing the dose of MLE, therefore the highest level of MLE (1:20) were the most effective in this regard. The differences between the three doses of MLE were significant at both cuts of the two seasons.

The effect of interaction treatments between NPK levels and MLE doses, the data indicated that all the concentrations of MLE under the same NPK level increased the TPHC comparing to NPK fertilized plants without MLE spray, NPK treatment at 100% with the highest concentration of MLE (1:20) had more promoting effect on increasing the TPHC in dry herb of *M. citriodora* L. plant. Also, the used of MLE at high dose 1:20 with NPK at 75% produced the same value of the plant treated with 100%NPK without spray MLE.

4. Total flavonoids content (TFC) mg/g

Comparing the effect of different levels of NPK on the TFC in extract of dry herb of *M. citriodora* plant, treatment of NPK at 100% had more promoting effects in increasing the total content of flavonoids. On the contrary, 50% NPK treatment gave the lowest value in this concern. The mean values were (5.18, 7.85 mg/g), (4.51, 6.96 mg/g) and (3.90, 6.05 mg/g) for 100%, 75% and 50% at the first and second cuts of the first season, respectively. While, in the second season, the values of TFC were (5.26, 8.58 mg/g), (4.62, 7.64 mg/g) and (3.98, 6.53 mg/g) for the same treatments respectively. Our results are in harmony with the findings of Osuagwu and Edeoga [46] on *Ocimum gratissimum* and *Gongronema latifolium* plants, Borella *et al*. [47] on *Baccharis trimera* and Barbara [48] on *Solidago virgaaurea* L., they found that mineral fertilization (NPK) increased the total flavonoids content**.**

Data in Table (4), indicate that foliar spraying of MLE significantly increased the TFC compared with control treatment in both cuts during the two seasons. The maximum mean values of TFC was recorded with applying the highest concentration (1:20) compared to other concentrations in the two seasons at both cuts. The mean values of total flavonoids with MLE at dose 1:20 were (5.20, 7.86) and (5.28, 8.44) mg/g against (3.89, 6.09) and (4.15, 6.65) mg/g for untreated plants which gave the least values in this respect at the two cuts during two seasons, respectively.

The interaction between NPK treatments and doses of MLE showed that, NPK at high level 100% with MLE at high dose 1:20 was the most effective treatment on the accumulation of total flavonoids content. On the other side, the treatment of 75% NPK plus spraying with MLE at high dose (1:20) resulted the same values of TFC of plants treated with NPK at 100% without spraying MLE. Also, the plants treated with 50% NPK plus application of MLE at high dose were more effective for increasing the TFC produced values about the same of NPK at 75% without used MLE.

Table 3. Effect of moringa leaves extract, chemical fertilizers and their interactions on total phenolic content (mg/g) of *Monarda citriodora* L. plants

				Total phenolic content (mg/g)				
				First season				
	First cut				Second cut			
Ext. Conc.	Chemical fertilizer rates (NPK)							
	100%	75%	50%	Mean	100%	75%	50%	Mean
Control	10.79	9.88	8.43	**9.70**	13.30	12.09	10.12	**11.84**
M1 (1:40)	11.64	10.66	9.87	**10.72**	14.55	13.14	10.92	**12.87**
M2 (1:30)	12.62	11.36	10.01	**11.33**	15.65	14.42	12.23	**14.10**
M3 (1:20)	13.11	12.09	10.71	**11.97**	16.78	15.05	13.40	**15.08**
Mean	**12.04**	**11.00**	**9.76**		**15.07**	**13.68**	**11.67**	
LSD(0.05)	Chem.			**0.55**				**1.21**
	Ext.			**0.23**				**1.48**
	Chem. X Ext.			**N.S.**				**N.S.**
				Second season				
Ext. Conc.	First cut				Second cut			
	100%	75%	50%	Mean	100%	75%	50%	Mean
Control	10.70	9.42	8.86	**9.66**	15.05	12.46	11.09	**12.87**
M1 (1:40)	11.29	9.76	9.42	**10.16**	15.24	13.35	11.32	**13.30**
M2 (1:30)	12.67	10.36	9.68	**10.90**	15.71	15.17	13.33	**14.74**
M3 (1:20)	13.14	11.39	10.12	**11.55**	16.61	15.57	13.79	**15.32**
Mean	**11.95**	**10.23**	**9.52**		**15.65**	**14.14**	**12.38**	
LSD(0.05)	Chem.			**0.09**				**0.34**
	Ext.			**0.10**				**0.24**
	Chem. X Ext.			**0.17**				**0.41**

Ext.Con. (Extract Concentration) M (Moringa leaves extract) Chem.(Chemical fertilizer rates, NPK) N.S. (Non-significant)

Table 4. Effect of moringa leaves extract, chemical fertilizers and their interactions on total flavonoids content (mg/g) of *Monarda citriodora* L. plants

				Total flavonoids content (mg/g)				
				First season				
	First cut				Second cut			
Ext. Conc.	Chemical fertilizer rates (NPK)							
	100%	75%	50%	Mean	100%	75%	50%	Mean
Control	4.31	3.88	3.47	**3.89**	6.86	6.22	5.20	**6.09**
M1 (1:40)	4.93	4.16	3.80	**4.30**	7.33	6.63	5.92	**6.63**
M2 (1:30)	5.39	4.79	4.04	**4.74**	8.25	7.11	6.33	**7.23**
M3 (1:20)	6.09	5.20	4.30	**5.20**	8.96	7.88	6.73	**7.86**
Mean	**5.18**	**4.51**	**3.90**		**7.85**	**6.96**	**6.05**	
LSD(0.05)	Chem.			**0.21**				**0.17**
	Ext.			**0.17**				**0.14**
	Chem. X Ext.			**0.3**				**0.25**
				Second season				
Ext. Conc.	First cut				Second cut			
	100%	75%	50%	Mean	100%	75%	50%	Mean
Control	4.71	4.15	3.60	**4.15**	7.88	6.54	5.54	**6.65**
M1 (1:40)	4.83	4.33	3.73	**4.30**	8.40	7.32	6.23	**7.32**
M2 (1:30)	5.56	4.76	3.91	**4.74**	8.82	8.09	6.87	**7.93**
M3 (1:20)	5.95	5.22	4.68	**5.28**	9.20	8.62	7.49	**8.44**
Mean	**5.26**	**4.62**	**3.98**		**8.58**	**7.64**	**6.53**	
LSD(0.05)	Chem.			**0.29**				**0.32**
	Ext.			**0.22**				**0.26**
	Chem. X Ext.			**N.S.**				**N.S.**

Ext.Con. (Extract Concentration) M (Moringa leaves extract) Chem.(Chemical fertilizer rate, NPK) N.S. (Non-significant)

5. Free radical scavenging activity (%)

Data in Table (5) reveal that the percentage of antioxidant activity was enhanced by increasing of NPK fertilization. The highest percentage of antioxidant activity (56.93%, 59.21%) and (58.29%, 58.70%) were obtained by using RD of NPK (100%), followed by75% NPK treatment (50.54%, 53.71%) and (53.88%, 52.14%), and then 50% NPK which produced the least values in this concern (42.64%, 45.55%) and (48.06%, 46.27%) in the first and second cuts of the two seasons ,respectively. The differences between the three levels of NPK were significant in both cuts during two seasons.

Comparing the effects between the different doses of natural extract (MLE), the data in Table (5) show that the three doses of MLE significantly increased the antioxidant activity % compared with untreated plants. These increments were gradually with increasing the dose of MLE. Therefore the highest dose of MLE (1:20) was the most effective in this regard. The differences between the three doses for both MLE were significant.

The interaction between various levels of both chemical fertilization (NPK) and natural extract concentrations (MLE), the results in Table (5), clear that under the same level of NPK, application of MLE increased the percentage of free radical scavenging activity comparing to the plants fertilized by NPK without spraying the natural extract. The optimum treatment was the plants fertilized by NPK at RD (100%) with raising the concentrations of MLE up to (1:20). The trend of these results was similar during the two experimental seasons.

Table 5. Effect of moringa leaves extract, chemical fertilizers and their interactions on antioxidant activity (%) of *Monarda citriodora* L. plants

	Antioxidant activity (%)							
	First season							
	First cut				Second cut			
Ext. Conc.	Chemical fertilizer rates (NPK)							
	100%	**75%**	**50%**	**Mean**	**100%**	**75%**	**50%**	**Mean**
Control	50.51	42.79	34.27	**42.52**	47.06	43.33	35.31	**41.90**
M1 (1:40)	55.92	47.86	38.92	**47.57**	60.18	53.13	43.90	**52.40**
M2 (1:30)	59.75	54.03	44.24	**52.67**	63.80	56.51	47.53	**55.95**
M3 (1:20)	61.54	57.48	53.13	**57.38**	65.80	61.88	55.46	**61.05**
Mean	**56.93**	**50.54**	**42.64**		**59.21**	**53.71**	**45.55**	
	Chem.			**1.48**				**1.21**
LSD(0.05)	**Ext.**			**1.35**				**1.02**
	Chem. X Ext.			**2.34**				**N.S.**
	Second season							
Ext. Conc.	**First cut**				**Second cut**			
	100%	**75%**	**50%**	**Mean**	**100%**	**75%**	**50%**	**Mean**
Control	47.45	43.22	38.06	**42.91**	51.69	45.60	39.48	**45.59**
M1 (1:40)	58.87	54.25	47.54	**53.55**	57.96	49.13	45.29	**50.79**
M2 (1:30)	62.02	57.87	51.96	**57.28**	61.70	54.68	49.13	**55.17**
M3 (1:20)	64.82	60.18	54.68	**59.89**	63.46	59.13	51.18	**57.92**
Mean	**58.29**	**53.88**	**48.06**		**58.70**	**52.14**	**46.27**	
	Chem.			**1.61**				**1.61**
LSD(0.05)	**Ext.**			**0.90**				**1.83**
	Chem. X Ext.			**1.56**				**N.S.**

Ext.Con. (Extract Concentration) M (Moringa leaves extract) Chem.(Chemical fertilizer rates, NPK) N.S. (Non-significant)

In general, foliar spray of *Monarda citriodora* L. plants with MLE at all different doses (1:40, 1:30 and 1:20) resulted gradual steady increase in the essential oil, total phenolic, total flavonoid content and antioxidant compared to control (without extract). Also, from the above mentioned results, it was observed that the used of the natural extracts interacting with low levels of chemical fertilization lead to increase the content of these characters compared to plants fertilized with higher levels of the chemical fertilization (NPK) without spraying the natural extract (MLE). So, MLE as foliar application can be reduce the chemical fertilizer dosage and to produce high quality of plants.

Similar results were obtained by El-Rokiek *et al*. on *Narssius tazetta* L. [29], Sakr *et al*. on *Pelargonium graveolens* L. [34] and Ali *et al.* [32] they proved that The MLE level applied 1:20 increased volatile oil content, total phenolic contents and radical scavenging activity of geranium leaves plant.

Discussion

Secondary metabolites contents such as essential oil, phenolic and flavonoids compounds as well as antioxidant activity depends on many variations factors. The chemical fertilizers stimulate the plant through the role of nitrogen, phosphorous and potassium in many physiological processes in the synthesis of many components of the plant (enzymes, protein, amino acids, nucleic acids, lipids….ex.) to increase the active constituents in plants. [49, 50].The nutrient levels such as N, P and K may alter the phenolic concentration in plants. For example, lower N rates lead to lower phenolic synthesis and antioxidant activity in basil. This may be the result of according to the carbon/nitrogen (C/N) and the limited of nutrient availability which determines the accumulation of secondary metabolites [51-53].

The positive effect of using MLE, on the chemical content of *Monarda citriodora* L. plant. These results may be due to that (MLE) provides an excellent source of bioactive compounds such as micro and macro elements (calcium, phosphorus, potassium, sulfur and iron and sodium) ,amino acids, phenolics, flavonoids, vitamins (A, B1, B2, B3, C, E), sugars, antioxidants, ascorbic acid, soluble sugars, thiamine, riboflavin , nicotinic acid, ascorbic acid, carotene and it is rich in phytohormones such as indole-3-acetic acid, gibberellins and zeatin a naturally-occurring cytokinin [54,55]. Growth hormones, especially zeatin, and sufficient quantities of the necessary nutrients with appropriate ratio which increase growth, yield components and the productivity of many plants [56].The MLE have several content of different elements such as Mg that would responsible for induction and increase in the amounts of chlorophyll a, b and contents of carotenoids (α, β carotene, lutein and xanthin) that have antioxidant properties [57-59].

The positive effects of NPK rates, natural extract of MLE and their interactions, may be due to the important physiological role of NPK in plants .Also, natural extract of MLE plays a key role in improving the volatile oil percentage. It caused significantly increased in photosynthetic pigments, caused accumulations of total sugars, promotion of cell division, cell elongation, chlorophyll biosynthesis and it can be considered as a beneficial solution to help the plants to overcome the harmful effects of environmental stress that reflected on an increase in volatile oil percentage and chemical contents of *Monarda citriodora* L. plant [59-61].

Conclusion

It may be concluded that chemical fertilizer plus foliar spraying with moringa leaves extract had a positive significant effect on the volatile oil production as well as chemical constituents (Total phenolic, Total flavonoids and Antioxidant activity). Interacting NPK at 75% with natural extract (MLE) at high dose (1:20), recorded increases in the Chemical constituents and essential oil production as compared to fertilized plants NPK 100% (RD) without extract spray. Also, NPK at 50% with high dose of MLE improved these characters compared to 75%NPK without MLE. So, for avoiding the excessive use of the mineral chemical fertilizers, it could be used MLE as natural bio stimulants.

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